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Exogenous β-mannanase improves feed conversion efficiency and reduces somatic cell count in dairy cattle

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ABSTRACT

Exogenous fibrolytic enzymes have been shown to be a promising way to improve feed conversion efficiency (FCE). β-Mannanase is an important enzyme digesting the polysaccharide β-mannan in hemicellulose. Supplementation of diets with β-mannanase to improve FCE has been more extensively studied in nonruminants than in ruminants. The objective of this study was to investigate the effects of β-mannanase supplementation on nutrient digestibility, FCE, and nitrogen utilization in lactating Holstein dairy cows. Twelve post-peak-lactation multiparous Holstein cows producing 45.5 ± 6.6 kg/d of milk at 116 ± 19.0 d in milk were randomly allotted to 1 of 3 treatments in a 3 × 3 Latin square design with 3 periods of 18 d (15 d for adaptation plus 3 d for sample collection). All cows were fed the same basal diet and the 3 treatments differed only by the β-mannanase dose: 0% dry matter (DM; control), 0.1% of DM (low supplement, LS), and 0.2% of DM (high supplement, HS) supplemented to the basal diet. Supplementation of β-mannanase enzyme at the LS dose reduced dry matter intake (DMI) but did not affect milk yield or milk composition. Cows receiving LS produced 90 g more milk per kg of DMI compared with control cows. Somatic cell count (SCC) in milk was lower for cows fed the LS diet compared with cows fed control diets. Cows fed LS diet had lower DM, organic matter and crude protein digestibility compared with cows fed control diets. Starch, neutral detergent fiber, and acid detergent fiber digestibility were not affected by LS. Milk yield, DMI, SCC, and nutrient digestibility did not change for HS. Despite the reduced crude protein digestibility, reduced N intake led to similar fecal N excretions in LS cows and control cows (234 vs. 235 g/cow per day). Urinary N excretions remained similar between enzyme-fed and control cows (~190 g/cow per day), although the percentage of N intake partitioned to urinary N tended to be greater in LS than in control cows (31 vs. 27%). Cows fed LS significantly improved the percentage of apparently absorbed N partitioned to milk protein N (42 vs. 38%). When supplemented at 0.1% of dietary DM, β-mannanase can improve FCE and lower the SCC of dairy cows without affecting milk yield, milk composition, or total manure N excretions of dairy cows.

Key words: β-mannanase, feed conversion efficiency, lactating cows, somatic cell count

INTRODUCTION

Improving feed conversion efficiency (**FCE**), which is the amount of product (e.g., milk yield) per unit of feed consumed, has a positive economic and environmental impact on a dairy enterprise. For example, within certain limits, greater inclusion of nonstructural carbohydrates in diets is often associated with greater milk yield per unit of DMI compared with diets with greater structural carbohydrates (Lascano et al., 2011). Increasing intake of available energy through improvement of fiber digestibility by exogenous fibrolytic enzymes can improve FCE. Although a considerable number of studies have shown increased milk production and ADG in ruminants, others did not observe improvement in FCE by using exogenous fibrolytic enzymes (Beauchemin et al., 2003). Exogenous xylanases and cellulases are the most commonly used fibrolytic enzymes for ruminants (Mendoza et al., 2014). Exogenous xylanase have been shown to increase total-tract digestibility of DM, NDF, and ADF in cattle (Yang et al., 2000), indicating that endogenous enzyme secretions in the rumen could be limiting, inefficient, or both, in attacking the substrate polysaccharides as target glycosidic bonds can be inaccessible to the active site of the enzymes (Boraston et al., 2004).

The polysaccharide β-mannan is an important component of the plant cell walls and can be classified into 4 groups: pure mannan, glucomannan, galactomannan, and galactoglucomannan (Moreira and Filho, 2008). Each of these plant cell wall polysaccharides present a

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backbone of β-1,4-linked mannose and glucose residues substituted with side chains of α-1,6-linked galactosyl side groups (Dea and Morrison, 1975). In contrast, a series of oligosaccharides containing α-1,2, α-1,3, and α-1,6 linkages have been isolated from yeast mannan. The mannosidic bonds in the β-mannans are hydrolyzed by β-mannanases found in glycoside hydrolase (**GH**) families 5 and 26 (Tailford et al., 2009), which also include cellulases and xylanases. Moreover, β-mannanases use mechanisms similar to those used by celluloses and xylanases in identifying and accessing the substrate (Sunna et al., 2001; Boraston et al., 2004). Hydrolysis of the β-mannans by β-mannanase releases mannan-oligosaccharides (**MOS**) of various lengths (Franco et al., 2004). Dietary supplementation of MOS has been shown to improve gastrointestinal health or overall health and performance of broilers (Yang et al., 2008), dogs (Swanson et al., 2002), and dairy calves (Heinrichs et al., 2003). Feeds containing high concentrations of β-mannan are palm kernel meal (30–35% of DM), palm kernel expeller (24% of DM), soybean hull (8% of DM), soybean meal (2% of DM) and sesame meal (3% of DM) (Dierick, 1989; Mok et al., 2013).

Saenphoom et al. (2013) reported the hemicellulose content of palm kernel expeller to decrease by 26% after treatment with β-mannanase. Lawal et al. (2010) reported that β-mannanase extracts from *Aspergillus niger* significantly increased hemicellulose digestibility in broilers fed palm kernel cake-based diets. Daskiran et al. (2004) reported that β-mannanase improved feed to gain ratio in broilers fed diets with soybean meal and guar gum. The effect of β-mannanase on FCE increased as guar gum content increased (Daskiran et al., 2004). Supplementation of β-mannanases has been shown to improve gain to feed ratios in pigs and broilers (Pettey et al., 2002; Kong et al., 2011; Cho and Kim, 2013; Lv et al., 2013) and feed digestibility in broilers, layers, and pigs (Wu et al., 2005; Kong et al., 2011; Mussini et al., 2011; Kim et al., 2013; Mok et al., 2013). However, greater FCE is not always related to digestibility improvements. Gharaei et al. (2012) and Zangiabadi and Torki (2010) demonstrated improvements in immune status parallel to increasing FCE in broilers for β-mannanase supplementations.

There is a paucity of information on β-mannanase produced by rumen bacteria. Nakai et al. (1994) examined degradation of β-mannan in the cultures of 26 strains of 9 species of rumen bacteria and found only 5 strains that degraded more than 20% of the β-mannan in culture media. About 80% of the strains were *Butyrivibrio fibrisolvens*. Fernando et al. (2010) showed that ruminal *Butyrivibrio fibrisolvens* populations declined by about 10-fold as the forage to concentrate ratio changed from 100:0 to 60:40 in cattle. Therefore, exogenous β-mannanase may enhance degradation of β-mannan in the rumen, when cows are fed total mixed rations containing high-mannan feeds such as palm kernel meal and soybean hulls. However, a recent study by Lee et al. (2014) showed no changes in total-tract CP or NDF digestibility in 6-mo-old goats for β-mannanase supplementation, even though it improved ADG, FCE, and N retention. Arndt et al. (2015) demonstrated that dairy cows with greater FCE were associated with lower SCC and greater N partitioning to milk protein compared with cows with lower FCE. The objective of this study was to investigate the effects of exogenous β-mannanase supplementation on nutrient digestibility, FCE, SCC, and N partitioning in lactating dairy cows.

MATERIALS AND METHODS

Animals and Treatments

All animal procedures were approved by Institutional Animal Care and Use Committee at the University of California-Davis. The experiment was conducted at the Teaching and Research Facilities of the Department of Animal Science at the University of California-Davis. Twelve post-peak-lactation multiparous Holstein cows with an average of 696 ± 47 kg of BW, 45.5 ± 6.6 kg/d of milk yield, and 116 ± 19.0 DIM at the beginning of the experiment were housed in a freestall barn equipped with Calan gates (American Calan, Northwood, NH). Cows were assigned to 3 treatments in a 3-period crossover design, where treatment sequences were balanced using 3 × 3 Latin squares to mitigate possible carryover effects. The treatments were a basal TMR diet only or supplemented with 2 doses of a commercially available β-mannanase enzyme (CTCZYME, patent 100477456–0000; CTC Bio Inc., Seoul, South Korea). The CTCZYME product contains pure β-mannanase, which is produced using a gene encoding mannanase of *Bacillus subtilis* WL-7 cloned into *Escherichia coli*. The mannanase gene encodes a polypeptide of 362 amino acids, the sequence of which is highly homologous to those of mannanases belonging to GH family 26. Kweun et al. (2004) reported that the enzyme had a pH optimum at 6.0 and a temperature optimum at 55°C. The activity of the enzyme was estimated to be 800,000 U/kg at pH 4.0 and 30°C (Kim et al., 2013). The enzyme was active on mannan sources such as locust bean gum, konjak, and guar gum, and it did not exhibit activity toward yeast mannan, carboxymethylcellulose, β-glucan, or xylan. The CTCZYME β-mannanase was previously tested in broilers (Kong et al., 2011; Mussini et al., 2011; Ferreira et al., 2016), pigs (Yoon et al., 2010; Kim et al., 2013), goats (Lee et al., 2014), and growing heifers (Seo et al., 2016). The enzyme product is available in

powder form and is not soluble in water. In the present study, the β-mannanase enzyme was supplemented at 0 (control), 0.1% of DM (low supplementation rate, **LS**), and 0.2% of DM (high supplementation rate, **HS**). The doses were determined based on the study by Lee et al. (2014), who demonstrated improved FCE at 0.06 and 0.17% of DM in 6-mo-old goats.

Ingredient and chemical composition of the basal diet are given in Table 1. The enzyme was offered to the animals by hand mixing it (~27 to 54 g/cow per day) thoroughly with corn silage (~20 kg as-fed/cow per day) first and then mixing the corn silage with rest of the feed. Mixing β-mannanase in a similar manner; that is, without using water as a carrier, has been shown to improve animal performance previously (Lee et al., 2014; Seo et al., 2016). Each experimental period lasted 18 d, consisting of 14 d for adaptation in the freestall barn, 1 d for adaptation to metabolic stalls, and 3 d for total collection of urine and feces from cows housed individually in metabolic stalls. Each metabolic stall was equipped with a feed trough, a water cup, and a rubber floor. Cows in the freestall barn and metabolic stalls were fed twice daily at 0800 and 2000 h at 105% of previous day intake. Milk yield and composition and DMI of individual animals were measured in both the freestall barn and metabolic stalls. All animals had free access to water at all times.

Sample Collection and Analysis

Feed offered to and refused by individual cows was weighed and recorded daily. Orts from individual cows were sampled daily in each period. Individual feed ingredients were sampled just before (on d 13) and at the end (on d 18) of sampling in each period. Feed ingredients and orts samples of individual cows were pooled by days within each period and stored at –20°C until analyzed for chemical composition. Cows were milked twice daily at 0600 and 1800 h. Milk samples were collected daily from individual cows and stored at 4°C until analyzed for milk fat, protein, lactose, MUN concentrations, and milk SCC (Central Counties DHIA, Atwater, CA).

Daily feces and urine output by individual cows (kg/d) in metabolic stalls were measured on d 16 through d 18 as described by Niu et al. (2016). We assumed that digestion and metabolic responses to enzyme would reach homeostasis after a 2-wk adaptation period. A similar procedure described by Niu et al. (2016) was able to capture most of the expected digestibility and N partitioning response changes for dietary modifications when analyzed with mixed-effect models accounting for variability of individual cows (nested in treatment sequence). Feces from individual cows were scraped with long hoe scrapers into a plastic tray assigned to each cow before they stepped or rested on it. The individual feces trays were weighed and a representative feces sample (100 to 150 g) was drawn every 3 h during the metabolic trial period. Fecal samples pooled within each day of each period were stored at –20°C until analyzed for chemical composition. Urine from each cow was collected using an indwelling Foley catheter (24 French, 75-mL balloon; C. R. Bard, Covington, GA) connected to a ~2- to 3-m-long Tygon tube (Fisher Scientific, Waltham, MA) running into a sterile plastic jar (Fisher Scientific). Urine catheters were installed immediately after cows had been moved to metabolic stalls (0800 to 1000 h). Sample collection began after a 24-h acclimatization period. Cows were calm with no obvious vocalizations or restlessness after the catheters had been installed. All urine cans were weighed, sampled, and emptied every 6 h (0800, 1400, 2000, and 0200 h). A 5-mL aliquot of urine was pipetted into a 250-mL plastic bottle containing 180 mL of 0.5 mol/L sulfuric acid at each time of sampling. Acidified urine samples were stored at –20°C until analyzed for total N concentration. Feed ingredients and orts samples were analyzed for DM (135°C, AOAC International, 2000; method 930.15), CP (6.25 × Kjeldahl N, AOAC International, 2000; method 990.03), NDF (Van Soest et al., 1991), ADF (AOAC International, 2000; method 973.18), lignin (Goering and Van Soest, 1970), starch (Hall, 2009, with correction for free glucose), total ash

Table 1. Ingredients and chemical composition of the basal experimental diet (% of DM)

Item	Value
Ingredient	
Corn silage[1]	33.2
Alfalfa hay[2]	26.9
Steam-flaked corn	10.4
Distillers grain	6.2
Soybean meal	6.2
Rolled barley	4.1
Mineral mix	1.3
Soybean hulls	7.3
Cotton seed	4.1
Calcium carbonate	0.10
Salt (NaCl)	0.12
β-Mannanase	0.0
Chemical composition	
DM	68.1
CP	16.8
ADF	24.6
NDF	35.9
Lignin	4.4
Starch	20.2
Ether extract	4.0

[1]Contained 46% DM and 8.1% CP, 39.9% NDF, and 30.9% starch on DM basis.

[2]Contained 87.3% DM and 24% CP, 33.8% NDF, and 2.8% starch on DM basis.

(535°C, AOAC International, 2000; method 942.05), and individual mineral contents (AOAC International, 2000) at Cumberland Valley Analytical Services Inc. (Maugansville, MD). Dried and ground fecal samples (1.0-mm screen) were analyzed for the same chemical composition as the feeds at Cumberland Valley Analytical Services Inc. The urine samples were analyzed for total N (Chen et al., 1992) at Penn State University (State College, PA).

Calculations and Statistical Analysis

Apparent total-tract digestibility (ATTD, %) of DM, OM, CP, NDF, ADF, and starch were calculated using fecal output (F, kg/d) and corresponding intake (I, kg/d) of the nutrient:

$$\text{ATTD} = (\text{I} - \text{F}/\text{I}) \times 100.$$

The nutrient intake was calculated by accounting for offered feed DM (OF, kg/d) and concentration of the nutrient in offered feed (OFc, % DM), and refused feed DM (RF, kg/d) and concentration of the nutrient in refused feed (RFc, % DM):

$$\text{I} = (\text{OF} \times \text{OFc}/100) - (\text{RF} \times \text{RFc}/100).$$

Apparently absorbed CP and OM flows (kg/d) were calculated by taking the difference between corresponding intake and fecal output. Feed conversion efficiencies were expressed as milk yield to DMI ratio (milk:DMI), milk yield to apparently absorbed OM ratio, and milk protein yield to dietary CP intake ratio. Nitrogen partitioning was calculated by expressing N in feces, urine and milk, and retained N as a percentage of N intake.

Effects of β-mannanase supplementation on intake and digestibility, milk yield and composition, FCE, and N partitioning were determined using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) according to the following model:

$$\text{Y}_{\text{ijkl}} = \mu + \text{T}_{\text{i}} + \text{P}_{\text{j}} + \text{S}_{\text{k}} + \text{C}_{\text{l}}(\text{S}_{\text{k}}) + \text{e}_{\text{ijkl}},$$

where Y_{ijkl} = the response variable of interest, μ = overall mean, T_{i} = fixed effect of ith treatment (i = control, LS, and HS), P_{j} = fixed effect of jth period (j = 1 to 3), S_{k} = random effect of kth treatment sequence (k = 1 to 3), $\text{C}_{\text{l}}(\text{S}_{\text{k}})$ = random effect of lth cow nested in kth treatment sequence, and e_{ijkl} = random error assumed to be independent and identically distributed from a normal distribution with a mean of 0 and a variance of $\sigma^2\left[\sim N\left(0, \text{I}\sigma^2\right)\right]$. Period × treatment interactions were initially included and found to be nonsignificant, and were subsequently excluded in the final analyses. Pair-wise comparisons of treatments means (control vs. LS, control vs. HS, and LS vs. HS) were carried out with the Tukey-Kramer adjustment test. Somatic cell counts were log-transformed for use in the statistical analysis, as the absolute counts ($\times 10^3$/mL) were not normally distributed. The least squares means values were transformed back to absolute counts (as shown in tables). Statistical differences were declared at $P < 0.05$, and a tendency toward significance was considered at $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

Feed Intake, Milk Yield, and SCC

Dry matter intake, milk yield, and milk composition responses to β-mannanase supplementation are given in Table 2. Dry matter intake decreased significantly by 1.8 kg/d (23.3 vs. 25.1 kg/d; $P < 0.001$) at low dose (LS) but remained similar to control at high dose (HS). Regardless of the dose, cows receiving β-mannanase consumed less than cows not receiving the enzyme ($P = 0.001$). Reduced DMI was not observed in other studies that used the present β-mannanase enzyme product in broilers, pigs, goats, or growing heifers (Kong et al., 2011; Kim et al., 2013, Lee et al., 2014, and Seo et al., 2016, respectively). Milk yield, milk composition, and milk component yields did not differ significantly with enzyme supplementations. This is the first study to examine the effect of β-mannanase on milk production. Other exogenous enzymes such as xylanase also targeting hemicellulose polysaccharides did not affect milk yield or composition (Romero et al., 2016). Somatic cell count in milk decreased in cows fed the LS diet ($P = 0.043$) but not the HS diet. The majority of cells in SCC are white blood cells such as macrophages, lymphocytes, and neutrophils, which fight against udder infections and repair damaged tissues (Bradley and Green, 2005; Yu et al., 2011). Although SCC may not be as sensitive as bacterial culture in identifying infected quarters (Middleton et al., 2004), ease of measurement and its reasonably strong relationship with total viable bacterial counts in milk make SCC an effective udder health indicator (Kasikci et al., 2012). Although cows having SCC >200,000 cells/mL are usually considered to have subclinical mastitis, a recent study by Nyman et al. (2016) demonstrated that the SCC cut-off to classify cows as having intramammary infections can be as low as 74,000 cells/mL. At the cow level, SCC is a reduced representation of the true inflammation because infection occurs at the quarter level (Bradley and Green, 2005). Therefore, an average SCC decreasing from 72,000 to 59,000 cells/mL could indicate that

exogenous β-mannanase shifted cows from close to subclinical mastitis to a reasonably healthy status.

Somatic cell count in milk can vary significantly due to other factors such as stage of lactation and parity regardless of whether cows are infected (Sharma et al., 2011). Yu et al. (2011) indicated that the progressive increases of SCC when parity increased might be related to other mechanisms stimulating the immune system in the absence of infections. Besides innate immunity-related responses such as elevated neutrophil percentages and production of tumor necrosis factor-α (TNF-α) and IL-1β (Oviedo-Boyso et al., 2007), SCC are positively related to adaptive immunity-related production of IL-17 and IFN-γ by bovine T lymphocytes (Rainard et al., 2015, 2016). Although previous studies using the β-mannanase product used in the current study did not look into the effects on immune responses, supplementation of β-mannanase from other sources has been shown to have such an effect. Improvements in health status for supplementation of β-mannanase or MOS in nonruminant species have been reported widely. In a study using broiler chicks, Jackson et al. (2003) reported that supplementation of β-mannanase significantly increased weight gain and reduced both upper and lower coccidial lesion scores in birds infected with *Eimeria* sp. or *Clostridium perfringens*. Ibuki et al. (2014) reported that MOS (e.g., β-1,4-mannobiose) supplemented with mannanase-hydrolyzed copra meal exerted anti-inflammatory effects such as decreasing the expression of TNF-α, IL-1β, and IL-17 in the colon of pigs suffering from intestinal inflammation. Moreover, Che (2010) indicated that dietary inclusion of MOS in diets for pigs could maintain the host's disease resistance while preventing overstimulation of the immune system. Therefore, the observed reduction in SCC is likely linked to a situation where the immune system responded optimally to inflammation in the mammary gland as mediated by MOS liberated via β-mannanase-induced hydrolysis of β-mannan in cows. The hydrolysis of β-mannan may also play a role in controlling the immune responses. The cellular component of innate immune system (e.g., neutrophils and macrophages) identifies pathogens using distinct molecules; namely, pathogen-associated molecular patterns (**PAMP**) expressed on the surface of pathogens (Forsberg and Wang, 2006). Binding of these PAMP to receptors present on the surface of innate immune cells activate those cells (Forsberg and Wang, 2006) and may stimulate their proliferation (Egger et al., 1996). Because PAMP include sugar residues such as mannans, β-mannan in feeds such as soybean hull can create a false signal of the presence of pathogens in the gut, initiating unwarranted immune responses. The β-mannanase-induced hydrolysis of β-mannan can eliminate such a signal and the associated immune responses. No previous study has examined the effects of exogenous β-mannanase on health aspects of cattle but supplementation of MOS has been shown to enhance immune responses to rotavirus in dry cows (Franklin et al., 2005) and maintain BCS of early lactating beef cows (Linneen et al., 2014). Furthermore, excessive immune responses, particularly adaptive responses, represent significant energy expenditure in mammals (Cutrera et al., 2010). Therefore, cows with lower SCC could spend less energy for immune functions than cows with greater SCC. Overall, our results warrant further investigation into immune responses for β-mannanase supplementation, particularly in early lactation, when cows are generally under considerable immune and energy challenges.

Table 2. Effects of β-mannanase supplementation on DMI, milk production, and milk composition of dairy cows

Item	Treatment[1]			SEM
	Control	LS	HS	
DMI (kg/d)	25.1[a]	23.3[b]	24.3[ab]	0.66
Yield (kg/d)				
Milk	34.4	34	34.8	1.63
Milk fat	1.31	1.25	1.33	0.08
Milk protein	1.05	1.04	1.07	0.05
Milk lactose	1.67	1.64	1.68	0.10
Milk SNF	3.02	2.99	3.05	0.14
Milk composition				
Fat (%)	3.81	3.67	3.85	0.15
Protein (%)	3.07	3.07	3.09	0.07
Lactose (%)	4.82	4.83	4.81	0.06
SNF (%)	8.78	8.8	8.79	0.06
MUN (mg/dL)	15.1	14.4	14.6	0.83
SCC ($\times 10^3$/mL)	72[a]	59[b]	71[a]	20.0

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[1]Control = no supplementation; LS = low β-mannanase dose (0.1% of DM); HS = high β-mannanase dose (0.2% of DM).

Nutrient Digestibility and FCE

Mean apparent total-tract digestibility of nutrients, apparently absorbed DM and OM, and FCE of treatments are given in Table 3. Compared with control, digestibility of DM, OM, and CP significantly decreased in cows fed LS diet ($P < 0.05$) but remained unaffected by HS diet. The digestibility of NDF, ADF, and starch were not significantly affected by β-mannanase supplementation. In agreement with our study, ATTD of DM and OM decreased, whereas NDF digestibility was unaffected in 6-mo-old goats fed the same β-mannanase enzyme as used here at a dose of 0.06% of DM (Lee et al., 2014). Peters et al. (2015) also reported that supplementation of 1,4-β-glucanase and 1,4-β-xylanase did not affect ruminal and total-tract digestibility of NDF and ADF in lactating dairy cows. In line with the present study, the CTCZYME β-mannanase at

0.06% of DM reduced total-tract digestibility of CP in goats but supplementation at a greater dose (1.7% of DM) did not have an effect. The effect of exogenous enzyme on nutrient digestibility in ruminants can vary greatly based on type of diet (Beauchemin et al., 2003). Álvarez et al. (2009) observed differential effects of exogenous xylanase between different diets as the xylanase increased and decreased total-tract digestibility of CP in lambs fed alfalfa hay and ryegrass silage, respectively. Yoon et al. (2010) tested 4 doses of the β-mannanase enzyme and observed a quadratic effect on CP digestibility in pigs, although the mid-range doses improved digestibility. Kong et al. (2011) found CP digestibility to change in a similar manner in broilers. Overall, β-mannanase supplementation appeared to affect nutrient digestibility differentially in ruminant and nonruminant animals, although high doses offset the effects in both cases.

Reduced DMI and nutrient digestibility led to lower apparently absorbed OM (14.3 vs. 15.8 kg/d) and CP (AACP = 2.56 vs. 2.82 kg/d, Table 3) in cows receiving LS compared with control diet ($P < 0.001$). Nonetheless, cows receiving the LS diet had improved FCE by 90 g/kg of DMI (7% improvement; $P = 0.014$) compared with control cows because milk production was not compromised. Consistently, the β-mannanase product used here significantly improved FCE in growing heifers (Seo et al., 2016) and goats (Lee et al., 2014). The FCE improvement in goats was also achieved at the expense of DM and OM digestibility (Lee et al., 2014). Cows receiving LS used AACP more efficiently to synthesize milk protein compared with control cows (milk protein:AACP = 0.42 vs. 0.38, respectively). The AACP (2.56 kg/d) of LS cows is equivalent to an AACP of a diet with lower CP content (15.5 vs. 16.8%). Broderick (2003) demonstrated similar improvement in milk protein synthesis efficiency (milk protein: AACP = 0.45 vs. 0.41) without compromising milk yields (34 kg/d) as dietary CP content decreased from 16.5 to 15.0%. Improved efficiencies of LS cows can partly be associated with the possibility that reduced DMI and CP digestibility caused protein to be supplied close to requirement, avoiding extra energy expenditure for excreting excess N. There is a metabolic cost of 7.2 kcal of ME per g of excess N excreted as urea (NRC, 1989); therefore, cows receiving the LS diet could spare energy for milk production compared with control and HS cows, even though energy supply to those cows was less as DMI and digestibility decreased. The improved milk production efficiency can also be linked to a potentially lower immune response level in the mammary glands, indicated by the low SCC, that could have a favorable effect on energy efficiency (Cutrera et al., 2010). Banos et al. (2013) reported a negative relationship between milk:DMI ratio and the blood immune marker CD3, which is positively associated with SCC (Rivas et al., 2002). Moreover, Arndt et al. (2015) showed that cows with greater milk:DMI ratio had lower SCC than cows with lower FCE. Feed conversion efficiency and enzyme dose appeared to have a quadratic relationship in our study, as FCE increased with LS but remained similar to control with HS (Table 3). The quadratic effect resembles more of a "bell curve" so a study that included a wider range of doses could assist in determining the true shape of the relationship. Nonetheless, Yoon et al. (2010) showed a numerically quadratic relationship ($0.30 > P$-value > 0.20) between gain:feed ratio and CTCZYME β-mannanase dose (0, 200, 400, and 600 U/kg) in growing and finishing pigs. With a greater sample size, Lv et al. (2013) reported a significantly quadratic relationship between FCE of growing pigs and β-mannanase supplemented at the same 4 doses.

Table 3. Effects of β-mannanase supplementation on apparent total-tract digestibility and apparently absorbed fluxes of nutrients, and production efficiencies

	Treatment[2]			
Item[1]	Control	LS	HS	SEM
Digestibility (%)				
DM	65.5[a]	63.1[b]	65.4[a]	0.8
OM	66.5[a]	64.2[b]	66.5[a]	0.8
ADF	44.1	42.4	43.9	1.4
NDF	46.8	44.8	46.5	1.3
Ash	59.8[a]	44.7[b]	49.1[a]	1.3
CP	65.7[ab]	63.4[b]	66.6[a]	0.7
Starch	94.9	95	95.4	0.4
AAOM (kg/d)	15.8[a]	14.3[b]	15.4[a]	0.471
AACP (kg/d)	2.82[a]	2.56[b]	2.80[a]	0.081
Efficiency				
MY:DMI	1.38[a]	1.47[b]	1.43[ab]	0.05
MY:AAOM	2.20[a]	2.41[b]	2.29[ab]	0.08
Milk protein:CP intake	0.24[a]	0.26[b]	0.26[ab]	0
Milk protein:AACP	0.38[a]	0.42[b]	0.39[a]	0.01

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]AAOM = apparently absorbed OM; AACP = apparently absorbed CP; MY = milk yield.

[2]Control = no supplementation; LS = low β-mannanase dose (0.1% of DM); HS = high β-mannanase dose (0.2% of DM).

Nitrogen Partitioning and Phosphorus Excretion

Nitrogen intake and its partitioning to fecal, urinary, milk, and tissue N are shown in Table 4. Because of reduced DMI, cows receiving the LS diet had low N intake compared with cows in the control and HS treatments ($P < 0.001$). However, fecal N output of LS cows remained similar (~231 g/d) to those of control and HS cows as the CP digestibility was reduced. Although cows receiving β-mannanase excreted less urine, their urinary N concentrations were greater than that of control cows. Therefore, the total urinary N output

Table 4. Effect of β-mannanase supplementation on nitrogen (N) partitioning and phosphorus (P) excretion

Item	Treatment[1]			SEM
	Control	LS	HS	
N intake (g/d)	686[a]	643[b]	673[a]	17.3
Fecal N (g/d)	235	234	224	7.6
Fecal N (% of N intake)	34.3[ab]	36.6[a]	33.4[b]	0.7
Urinary N output (g/d)	185	199	195	10.5
Urinary N output (% of N intake)	26.9	31.3	29.1	1.7
Fecal N + urinary N (g/d)	419	432	419	14
Milk protein N + MUN (g/d)	170	168	172	7.7
Retained N (g/d)	97.0[a]	42.3[b]	81.0[ab]	14
Manure P (g/d)	77	79	75	2.4

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Control = no supplementation; LS = low β-mannanase dose (0.1% of DM); HS = high β-mannanase dose (0.2% of DM).

remained similar across all cows. Compared with cows in the control group, cows receiving LS tended to have greater urinary N excretions proportional to N intake ($P = 0.86$), even though they were expected to have less urinary N partitioning as they had incorporated more dietary N intake or apparently absorbed N to milk protein (Table 3). Perhaps β-mannanase supplementation reduced amino acid partitioning to tissue gain relative to the increased milk protein synthesis efficiency, as indicated by the less retained N of LS cows compared with control cows (Table 4). Bequette et al. (2001) demonstrated that infusion of insulin increased blood flow to the mammary glands compared with that to hind-leg in lactating dairy goats. β-Mannans, the substrates of β-mannanase, have been shown to inhibit insulin secretion in human (Morgan et al., 1985) and swine (Leeds et al., 1980). In the present study, a β-mannanase-induced insulin secretion might have enhanced amino acids uptake by the mammary gland compared with the other tissues. Thus, investigations into the relationships among β-mannanase, insulin, and N partitioning are warranted in future studies. Nonetheless, supplementation of β-mannanase did not change total N excreted in manure (g/cow per day), and did not affect manure phosphorus output, although exogenous β-mannanase has been shown to reduce manure phosphorus output in growing pigs (Lv et al., 2013). Adesogan et al. (2014) emphasized that giving animals a sufficiently long adaptation period is key to capturing the true effects of fibrolytic enzymes in ruminants. In our experiment, the majority of the responses remained unchanged over the sampling period (d 16 to 18), indicating that the responses to the enzyme supplementation had reached a steady state after 2 wk (data not shown). Potential carryover effects in crossover designs may make it more difficult to tease out true treatment effects. However, the nonsignificant period × treatment interactions (i.e., $P = 0.42$, 0.23, 0.63, and 0.32 for OM digestibility, milk yield, FCE and SCC, respectively; data not shown) indicated negligible carryover effects, given that the treatment assignments were balanced (Tempelman, 2004). Nevertheless, studies with continuous designs for longer periods need to be conducted to validate the results in this experiment.

CONCLUSIONS

Supplementation of β-mannanase to a corn silage and alfalfa hay-based TMR at a dose of 0.1% of DM increased milk yield per kilogram of DM and milk protein yield per kilogram of CP intake, and decreased SCC in milk of lactating dairy cows. Supplementation of β-mannanase at 0.2% of DM did not have a significant effect on any of these responses. Apparent total-tract digestibility of DM, OM, and CP decreased in response to the 0.1% DM dose but were not affected at the 0.2% dose. Dietary N partitioning to urinary N increased, whereas N retained in the body decreased only in response to the 0.1% DM dose. However, total manure N output (fecal N plus urinary N) was similar across cows with and without β-mannanase supplementation. Therefore, when supplied at a 0.1% of DM, β-mannanase was able to improve FCE and reduce SCC without affecting manure N excretion. The role of β-mannanase supplementation may be more critical in early lactating cows, which are generally under significant metabolic and immune challenges. Further research is needed to assess the mechanism and long-term effect of β-mannanase enzyme in lactating dairy cows.

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